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Research Article

Phytochemical Analysis and Antimicrobial Studies of Various Extracts of Tomato (*Solanum lycopersicum* L.)

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Abstract: Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components. Tomatoes contain a variety of phytochemicals such as lycopene, -carotene, vitamin C, quercetin glycosides, naringenin chalcone and chlorogenic acid and have good health protective effects. The present work is to search antimicrobial activity of methanol (E1),ethanol (E2),acetone(E3),chloroform (E4) and ether (E5) extracts from tomato fruits. The antibacterial property was evaluated by using agar diffusion method using bacterial cultures *Staphylococcus aureus* (ATCC 9144),*Bacilus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and fugal culture of *Aspergillus niger* (ATCC 9029), *Aspergillus flavus* (ATCC 204304), *Candida albicans* (ATCC 10231). By observing it was found that most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. The extracts were active against all the tested microorganism for anti-bacterial activity with range of MIC values for *S.aureus* (MIC: 15-39 µg /ml), *E.coli* (MIC: 16-38 µg /ml) ,*P.aeruginosa* (MIC:15-39 µg /ml) and *B.subtilis* (14-39 µg /ml) The extracts were active against all the tested microorganism for anti-fungal activity with the range of MIC values for *A.niger* (MIC :17-39 µg/ml),*A.flavus* (18-37 µg/ml) and *C.albicans* (16-35 µg/ml).
Keywords: *Solanum lycopersicum* L., Phytoconstituents, Lycopene, antibacterial activity, Antifugal activity

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components [1]. The consumption of tomatoes has been proposed to reduce the risk of several chronic diseases such as cardiovascular diseases and certain types of cancer and especially prostate cancer [2, 3]. In addition, tomato consumption leads to decreased serum lipid levels and low density lipoprotein oxidation [4]. Tomatoes contain a variety of phytochemicals, including carotenoids like lycopene(highest concentration -85%),phytoene, phytofluene and the provitamin A, carotenoid β-carotenoid, polyphenols including quercetin, kaempferol, naringenin, neutriants like folate vit-C, vit-E, vit-K vit-B, phosphorus,sulphur potassium calcium,iron (significant quantities),sugars like aldoses, ketoses, disaccharides, polysaccharides mainly starch, proteins and amino acids, enzyme polyphenol oxidase,phytosterol like cholesterol, sitosterol and small quantities of fats. All of these are known to contribute significantly to the antioxidant activity of tomato fruit [3, 5]. These health protective effects have been widely attributed to the presence of key antioxidants such as lycopene, -carotene, vitamin C, quercetin glycosides, naringenin chalcone and chlorogenic acid.

The number of infections which are caused by multi drug resistant gram positive and gram negative pathogens and viruses are life threatening for human being. Infections caused by these organisms pose a serious challenge to the scientific community and need for a effective therapy has lead for novel antimicrobial agents. The objective of the present work is to search antimicrobial activity of methanol (E1),ethanol (E2),acetone(E3),chloroform (E4) and ether (E5) extracts from tomato fruits.

MATERIAL AND METHODS

Drugs and Chemicals

Standard drug Ciprofloxacin, Ketoconazole were purchased from Local Retail Pharmacy Shop and Solvents and other chemicals were used from Institutional Store and were of analytical grade. Bacterial cultures *Staphylococcus aureus* (ATCC 9144),*Bacilus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and fugal culture of *Aspergillus niger* (ATCC 9029), *Aspergillus flavus* (ATCC 204304), *Candida albicans* (ATCC 10231) were obtained from Biotechnology Lab of the Institute and maintained on Nutrient agar slant and fungus strains were maintained on Sabouraud dextrose broth at 4^0C.

Extraction

Weigh 20 g of red tomato paste (ripe tomatoes can be mashed to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of methanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the

mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing lycopene with three portions of 150 ml each with sodium chloride solution. Dry the organic larger over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating.

The fruit paste of tomato were extracted with methanol (E1),ethanol (E2),acetone(E3),chloroform (E4) and ether (E5) and tested for phytochemical and antimicrobial activities.

Preliminary Phytochemical studies

Preliminary phytochemical studies for identification for reducing sugar, pentoses, hexose, disaccharides, starch, glycogen , proteins and amino acids, sterols, carotinoids, flavaoids, and polyphenols were performed using standard procedure [6,7].

Antimicrobial studies using Paper disc diffusion method

The antibacterial property was evaluated by using agar diffusion method [8,9]. The sterilized (autoclaved at 120^{o}C for 30 min) medium was inoculated (1mL/100mL of medium) with the suspension [10^5 cfu m/l (colony forming unit per milliliter)] of the microorganism (matched to McFarland barium sulphate standard) and poured in Petridish to give a depth of 3-4mm. The paper impregnated with the test compounds (50, 100,150 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1hr at RT and incubated at 37 ^{o}C for 24 hr for anti-bacterial and antifungal activities respectively. Ciprofloxacin (100 µg/disc) and Ketoconazoloe (100 µg/disc) was used as a standard.

MIC was determined by agar streak dilution method. A stock solution of the synthesized compounds (100µg/ml) in Dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the compounds was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately10^5 cfu m/l and applied to plates with serially diluted compounds in Dimethyl formamide to be tested and incubated at 37^{o}C for 24hr. for bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate. The observed MIC is represented in table.

RESULT AND DISCUSSION

The phytochemical analysis of various extracts was performed using standard procedure and have found following phytoconstituents given in **Table-1.**

Table-1: Phytochemical analysis of various extracts of Tomato (*solanum lycopersicum* L.)

Phytoconstituents	Presence in Extracts
Reducing sugars	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Pentoses	E1 (-), E2(-), E3(-), E4(-), E5 (-)
Ketohexose	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Disaccharides	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Monoaccharides	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Starch	E1 (+), E2(+), E3(+), E4(+), E5 (+)
Glycogen and dextrin	E1 (-), E2(-), E3(+), E4(-), E5 (-)
Aromatic amino acid	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Tyrosine	E1 (+), E2(+), E3(-), E4(-), E5 (-)
Typtophan	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Arginine	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Alpha amino acids and dipeptides	E1 (+), E2(+), E3(-), E4(-), E5 (-)
Sterol	E1 (-), E2(-), E3(-), E4(+), E5 (+)
Carotenoids	E1 (+), E2(+), E3(+), E4(-), E5 (-)
Poly phenols	E1 (-), E2(-), E3(-), E4(+), E5 (+)
Flavanoids	E1 (-), E2(-), E3(-), E4(+), E5 (+)

Methanol Extract(E1), Ethanol Extract(E2), Acetone Extract(E3),Chloroform Extract(E4) and Ether Extract(E5) Presence(+), Absence(-).

The observed zone of inhibition was compared with standard these observed zones of inhibition are represented in **Table-2** and **Table-3.**

The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate. The observed MIC is represented in **Table-4 and Table-5.**

Table-2: Zone Of Inhibition (mm) Of Bacteria

NAME OF THE EXTRACTS	***S. Auereus***			***E.coli***			***P.aeruginosa***			***B.subtilis***		
	CONCENTRATION (µg/disc)											
	50	100	150	50	100	150	50	100	150	50	100	150
E1	16	19	22	17	19	20	14	16	18	14	15	18
E2	19	20	23	20	22	23	19	20	22	17	19	21
E3	17	18	19	18	20	21	17	18	20	15	17	19
E4	18	19	20	19	21	22	18	19	21	16	18	20
E5	15	17	16	16	18	20	15	16	19	14	15	17
CIPROFLOXACIN (100µg/ml)	38	38	38	38	38	38	37	37	37	30	30	30

Methanol Extract(E1), Ethanol Extract(E2), Acetone Extract(E3),Chloroform Extract(E4) and Ether Extract(E5), Control: DMF , Standard – Ciprofloxacin

Table-3: Zone Of Inhibition (Mm) Of Fungi

NAME OF THE EXTRACTS	***A. niger***			***A. flavus***			***B. albicans***		
	CONCENTRATION (µg/disc)								
	50	100	150	50	100	150	50	100	150
E1	19	20	23	20	20	22	18	20	22
E2	18	19	21	19	19	21	17	19	21
E3	17	16	19	16	18	19	16	19	20
E4	20	22	24	21	22	23	19	21	23
E5	17	14	15	16	16	17	15	16	19
KETOKONAZOLE (100µg/ml)	38	38	38	35	35	35	36	36	36

Methanol Extract(E1), Ethanol Extract(E2), Acetone Extract(E3),Chloroform Extract(E4) and Ether Extract(E5), Control: DMF , Standard – Ketokonazole

Table-4: Minimum Inhibitory Concentration(MIC) of Extracts(Bacteria

EXTRACTS	MINIMUM INHIBITORY CONCENTRATION (µg/ml)			
	S. aureus	***E. coli***	***P. aerug***	***B. subt.***
E1	36	31	32	35
E2	15	20	29	31
E3	33	32	15	33
E4	17	16	39	14
E5	39	38	35	39
Ciprofloxacin	0.2	0.2	0.2	0.2

Table-5: Minimum Inhibitory Concentration(MIC) of Extracts(Fungi):

EXTRACTS	MINIMUM INHIBITORY CONCENTRATION (µg/ml)		
	A. niger	***A. flavus***	***C. albicans***
E1	**17**	**36**	**32**
E2	**35**	**31**	**16**
E3	**34**	**40**	**30**
E4	**25**	**18**	**21**
E5	**39**	**37**	**35**
KETOCONAZOLE	**6.1**	**6.1**	**6.1**

The various extracts posses both antibacterial and antifungal activity. These activities are due to presence of phytoconstituents mainly phenolic and sterols compounds. This may be the reason of Chloroform Extract(E4) and Ether Extract(E5) showed more antibacterial activities. Previous literature showed that phenolic compound showed good antibacterial activity [10].

Lycopene, the red pigment of tomato, is a tetraterpene assembled from eight isoprene units composed entirely of carbon and hydrogen, containing 11 conjugated and two nonconjugated carbon-carbon double bonds [11]. Various studies have shown that the consumption of a lycopene-rich diet reduces the incidence of cancers and heart diseases. Lycopene possesses antibacterial and antifungal properties [12, 13]. It is an effective adjuvant in the treatment of gingivitis along with oral prophylaxis [14]. Lycopene exerts potent antifungal activity against *Candida albicans* by causing significant damage to the cell membrane [15].

CONCLUSION

By observing it was found that most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. The extracts were active against all the tested microorganism for anti-bacterial activity with range of MIC values for *S.aureus* (MIC: 15-39 µg /ml), *E.coli* (MIC: 16-38 µg /ml) ,*P.aeruginosa* (MIC:15-39 µg /ml) and *B.subtilis* (14-39 µg /ml) The extracts were active against all the tested microorganism for anti-fungal activity with the range of MIC values for *A.niger* (MIC :17-39 µg/ml),*A.flavus* (18-37 µg/ml) and *C.albicans* (16-35 µg/ml).

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